Abstract No.: beau0199

Infrared Mapping of Functional Groups Involved in Metal Retention in Trout Livers Enriched in Zn Compared with a Control Liver

S. Beauchemin, J. Nadeau, and J. McGeer (Natural Resources Canada, Ottawa); S.-L. Wang (N.C. State Univ.), and N. Marinkovic (NSLS, Brookhaven National Laboratory)

Beamline(s): U10B, X11A

Introduction: The toxicological impacts of chronic metal exposure for aquatic biota are not well understood, partly due to the lack of understanding of metal speciation and complexation at a cellular and tissue level. In a related study, we characterized Zn speciation in the liver of rainbow trout (*Oncorhnychus mykiss*) exposed to Zn enriched water in relation to unexposed (control) fish using X-rays absorption spectroscopy (XAS). The objective of this IR study was to compare the changes in the main functional groups responsible for metal retention between the two treatments.

Methods and Materials: The blood was partially drained out of the livers prior to preparation for cryo-sectioning. The cryo-sectioning was performed by soaking the livers in sugar solutions followed by immersion in a cryomatrix (active ingredients: 10% polyvinyl alcohol and 4% polyethylene glycol). The livers were then cryo-cut into sections of 10 μm thickness. The cryo-cut sections were placed on Ag-coated microscopic plates, carefully rinsed 3 times with distilled water to wash out as much as possible the cryomatrix and left to dry. Synchrotron IR data were collected in reflectance mode using the MCT-A detector at the u10B beamline at the National Synchrotron Light Source, Brookhaven National Laboratory, NY. Maps of 17 x 12 pixels with a step size of 10 μm x 10 μm and a number of 64 scans by pixel were collected on each sample using the empty Ag-coated slide as the background.

Results: The Zn-exposed liver showed a small bioaccumulation of Zn compared to the control liver (22.32 vs 13.73 mg kg^{-1}, respectively; $p < 0.05$). XAS results indicated that the Zn speciation in livers from the Zn-exposed group was comparable to that of the control treatment, and was consistent with a Zn-sulfide bond (data not shown). Possible reduced S functional groups for metal retention would be thiol (-SH, e.g. cysteine) and disulfide (-S-S-, e.g. cystine). These S groups could not be resolved by our IR data. Apart from S, other functional groups for metal retention in organic materials would be carboxyl (-COOH), aldehyde (-COH), hydroxyl (-CHOH) and amine ($-NH_2$). Two main bands were consistently present in the region of 1800 – 1400 cm^{-1} associated with these groups, and are referred hereafter as amide I (1775 to 1608 cm^{-1}) and amide II (1610 to 1502 cm^{-1}) bands. The IR images were first processed by taking the area peak ratio of amide I or amide II bands to lipid band (2999 to 2805 cm^{-1}). The results showed more intense ratios for the Zn-enriched than the control liver (Fig. 1). However, IR maps obtained by taking the peak area of each component of the ratio indicated that the difference was mainly due to the lipid peak area, which was notably lower in the Zn-enriched treatment than in the control (Fig. 1). The IR maps of the peak areas for the two main bands in the region of 1800 – 1400 cm^{-1} showed that some areas in the tissues contained more amine, carboxyl and carbonyl groups than others. The surface of the liver section containing higher levels of these groups was greater in the control than in the Zn-exposed treatment (data not shown).

Conclusions: Overall, the Zn-enriched liver section contained less carboxyl, carbonyl and amine groups than the control liver. The lipid band area was also notably reduced in the Zn-enriched treatment. Future work needs to (i) evaluate whether the observed effects are related to artefacts during sample preparation or to real differences in the metabolism between the Zn-exposed fish and the control group, and (ii) concentrate on the identification of thiol and disulfide groups.

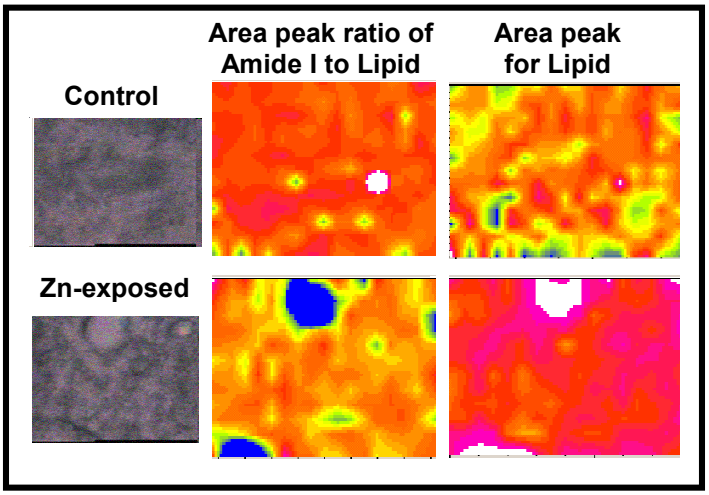

Figure 1. Visible (left) and IR images obtained by taking (i) the area peak ratio of Amide I to lipid band (middle) or (ii) the peak area of lipid band alone (right) for the control (top) and the Zn-exposed (bottom) treatments. Intensity scale ranges from pink - red (lowest) to blue (highest).